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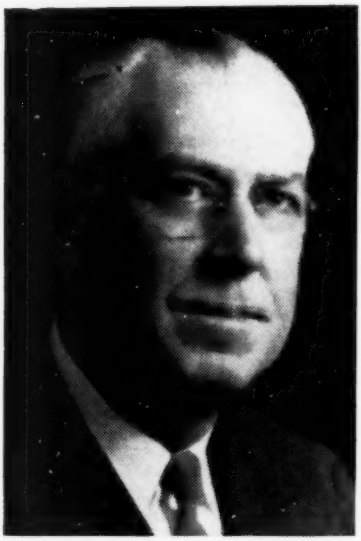

DR. GEORGE F. REDDISH

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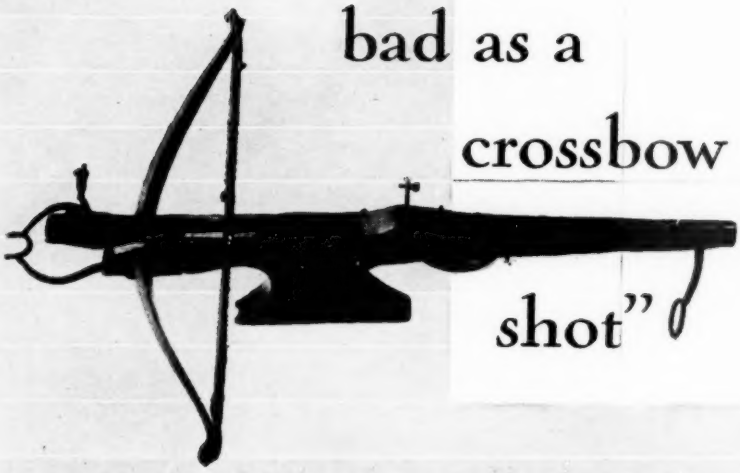

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EDITORIAL

TOBACCO—HABIT OR HAZARD?

ONE, today, must indeed be courageous to suggest that smoking is potentially harmful and not an unmitigated blessing. It is difficult for us to judge the virtues of tobacco with pure objectivity for the habit is so commonplace and so firmly entrenched in our social order that it is almost a part of man himself. Just recently another bit of evidence was submitted, adding to the already lengthy testimony against tobacco. It has been found that lung cancer is more frequent in heavy smokers than in non-smokers and that this increased frequency is statistically significant.

We shall not attempt to discuss all of the possible harm that may result from smoking for few would be interested in having one of their favorite diversions so assailed. There are, however, certain aspects of the problem that should be faced intelligently and honestly by those of us who are engaged in the health professions.

In the first place it is hard to reconcile some of the cigarette advertising appearing in certain medical journals with their otherwise rigid advertising policy. A journal that refuses advertising of many drug products on the basis that they are not yet sufficiently established therapeutically or that their names are not ethical can hardly justify advertisements of a product like cigarettes which is not just inert but actually potentially harmful. We also are somewhat dubious over the advertised results leading the reader to the conclusion that certain brands are less harmful than others. Far less questionable results concerning a pharmaceutical product would be condemned at once by our medical colleagues.

Few people stop to analyze certain of our "accepted" social graces such as smoking in order to determine in what manner they differ from "unaccepted" social practices. We wager that had the same billions been spent in popularizing and gaining approval of the use of marihuana over the past fifty years, as has been spent in promoting tobacco, marihuana smoking would be considered an entirely acceptable practice. In terms of human misery alcohol has

done more than cannabis ever could to destroy people's minds and bodies and yet cannabis is rigidly controlled by Federal legislation while alcoholic beverages are sold in quantities that stagger the imagination.

It is our opinion that the determining factor in these matters is the number of people involved rather than the basic harmfulness of the substance in question. This is surely not a scientific approach. Smoking is begun, usually at an early age, in order to copy the behavior patterns of those whom we highly regard—whether they be parents, motion picture heroes, or others. It is, at the beginning, quite unpleasant but persistence overcomes this and, finally, habituation develops. From then on we resent any effort to discourage us in our addiction and discount all evidence that it is harmful. Exactly the same sequence of events results from the use of alcohol, morphine, cannabis or any other drug commonly leading to addiction.

The former efforts of the cigarette manufacturers to convince the public that smoking was an entirely acceptable practice are interesting. We remember the days when it was almost unheard of to see a woman smoking in public. By a carefully planned program this concept has been changed. First only the more questionable ladies on the screen smoked during their parts and then ever so gradually the heroines were found to indulge. Later, when the public accepted this, even sweet, white haired grandmothers on the screen and on billboards were seen "lighting up." Needless to say, sales boomed as a result of such a well planned program. We cite this not because we feel that women are less entitled to smoke than men but only to show the power of propaganda in influencing our judgment.

Strange as it may seem we do not suggest for a moment the adoption of laws prohibiting the use of tobacco and alcohol. A life completely devoid of all such diversions undoubtedly would be drab and uninteresting. We, furthermore, admit our own indulgence in some of these addictions. We should, however, face these problems honestly and objectively and not let the great mass of advertising and public opinion alter our decision on such matters. The public has a right to expect from us an honest appraisal and not merely the reflection of its own opinion which is the product of propaganda and mass advertising.

It has been aptly stated before that "the best things in life are immoral, illegal or fattening" and this may well be true. We should not, however, allow our enthusiasm and participation in these things to tincture our judgment when called upon to give an honest opinion. Scientific and professional veracity demand an honest answer and a clear portrayal of the facts.

L. F. TICE

RECENT DEVELOPMENTS IN METHODS OF TESTING ANTISEPTICS*

By George F. Reddish, Ph. D.**

IT is good practice to review from time to time recent developments in specialized fields of science, especially those activities pertaining to pharmaceuticals. In doing so we should not only attempt to evaluate present information in relation to the past but we should also plan for the future. A discussion of the field, the testing of antiseptics, lends itself particularly well to such analysis.

Ten years ago, I attempted to do this in a study of methods of testing germicides (1). Newer developments in the field were evaluated and compared to established, recognized procedures. This study brought out the advantages and disadvantages of new techniques for the laboratory testing of germicides. But during the intervening ten years modifications of established methods have been suggested, as well as certain entirely new methods of testing. So it will now be my purpose to discuss these newer developments and to attempt to evaluate their importance in terms of practical application.

It is necessary to limit this discussion to methods of testing and their interpretation, for the simple reason that it would be practically impossible to include all new information relative to antiseptics within the scope of this review. As a matter of fact, a discussion of our present methods of testing—and their interpretation in the light of new developments in the field—is of sufficient importance to justify special attention.

Definition of Antiseptics

First, it will be necessary to define the word "antiseptic" as it is now used. This really should not be required but, since the word "antiseptic" is still being misused, a few brief definitions are in order.

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* An address made before the Eastern Scientific Section of the American Pharmaceutical Manufacturers' Association on Feb. 7, 1949.

** Professor of Bacteriology, St. Louis College of Pharmacy and Allied Sciences, and Director, Bacteriological Research, Lambert Pharmacal Company, St. Louis, Missouri.

Although the word "antiseptic" literally means "against sepsis" or "against infection," the word has been applied technically to mean substances which merely "inhibit the growth of bacteria." This wholly erroneous definition is still used in recent textbooks on bacteriology and in certain recently published papers on the subject.

It is only necessary to take the original meaning of the word, that is, "against infection," in order to arrive at the proper definition. Not only is this the correct definition, but it is the one used by the medical, dental, and veterinary professions and by the layman as well. This being the case, it is necessary that the correct definition be clearly understood and properly used by all manufacturers and users of antiseptics.

From a purely regulatory standpoint, it is important that the definition of antiseptics be clear. In a Supreme Court decision it was stated that words used on labels of products in interstate commerce should be interpreted in terms clearly understood by those to whom they are addressed. This important matter has received the official attention of the U. S. Food and Drug Administration. The following definition was first used by the F. D. A. in the control of antiseptics:

> "Antiseptics are substances which, when applied to microorganisms, will render them innocuous either by killing them or preventing their growth, according to the character of the preparation or the method of application. This term is used especially for preparations applied to living tissue." (2) (3)

Essentially the same definition has been included in the new Federal Food, Drug and Cosmetic Act (4) as follows:

> "The representation of a drug, in its labeling, as an antiseptic, shall be considered to be a representation that it is a germicide, except in the case of a drug purporting to be, or represented as, an antiseptic for inhibitory use as a wet dressing, ointment, dusting powder, or such other use as involves prolonged contact with the body."

A very careful study of the meaning of "antiseptic," "disinfectant," and related words was made independently by Patterson (5) in which the following definition of "antiseptic" is given:

> "*antiseptic,* n. A substance that opposes sepsis, putrefaction, or decay; one that prevents or arrests the growth or action of microoganisms, either by inhibiting their activity or by destroying them; used especially of agents applied to living tissue."

There is no valid reason for any misunderstanding as to the definition of this important word. Manufacturers, the Food and Drug Administration, the medical profession, and the layman all appear to understand what is meant by the term "antiseptic." So it is surprising that there should be any misunderstanding as to the real meaning and proper definition of this word.

If a drug acts "against infection," it can do so either by killing infectious microorganisms or by preventing their growth. The most certain means for acting "against infection" is to actually kill the bacteria present. Antiseptics which act in this manner are called germicides. If, on the other hand, a drug is of such a nature, or is used in such a way as to simply prevent the growth of bacteria, and by so doing prevent infection, they can also be and are classed as antiseptics. These latter are really bacteriostatic agents and not germicides, but they justify being classed as antiseptics because, when used clinically, they act "against infection." It is therefore not difficult, nor is it inconsistent, to give a double meaning to this word. Many English words have double meanings, and the word "antiseptic" is a good example.

Present Standard Methods of Testing Liquid Antiseptics

The present standard methods of testing antiseptics used in this country were designed to meet this double meaning. Products classed as antiseptic may be either germicidal or bacteriostatic agents. Our standard laboratory methods of testing are designed to test for both kinds of antiseptic activity.

In other words, our present standard methods of testing antiseptics are based on the definitions given. They were developed by the U. S. Food and Drug Administration (3) (6) and have been used for twenty-two years for the testing and control of this class of drugs. Although other procedures have been suggested from time to time, these methods, the F. D. A. methods, are still employed for the purpose.

Some attempts have been made recently to question the official nature of the U. S. Food and Drug methods of testing antiseptics and disinfectants. Such criticism has not met with success—and for good reason. The principal reason is that the U. S. Food and Drug Administration set forth these methods in an official publication (Circular 198, U. S. Dept. of Agriculture). Also, the then Secretary of Agriculture, in a signed statement dated October 15, 1931, specified that the methods set forth are *prescribed* for use in the examination of antiseptics and disinfectants. According to Webster's New International Dictionary (Second Edition, Unabridged) the word "prescribe" is defined as follows:

> "To lay down authoritatively as a guide, direction, or rule of action; to impose as a peremptory order; to dictate; direct; ordain; to keep within limits or bounds; to direct, designate, or order the use of; to give directions; Syn.—Limit, control, order, guide."

It is evident that in prescribing these methods the Secretary did so in an official letter and in his official capacity—that is, he directed, peremptorily ordered, dictated, ordained, designated, ordered the use of, and gave directions for the use of, these methods for the testing of antiseptics and disinfectants.

The official nature of these tests is further substantiated by the following statement in the official circular describing them:

> "Confusion has arisen from the fact that, in many cases, manufacturers have not used the same methods of testing their products, as a basis for preparing their labels, as those used by the administration. This possibility of misunderstanding would be obviated if the same methods were employed by all, and many manufacturers, recognizing this, have requested information as to methods employed by the Food and Drug Administration. In view of this, it seemed desirable to publish them in a form which would make them generally available.
>
> "This circular, therefore, describes briefly the methods usually employed in the insecticide control laboratory for testing official samples of antiseptics and disinfectants."

There is no doubt as to what this government official, the highest executive officer in the U. S. Department of Agriculture, meant in

this official statement accompanying methods of testing which were also officially designated the United States Food and Drug Administration Methods of Testing Antiseptics and Disinfectants (6). In addition, the F. D. A. method for testing liquid antiseptics is officially described as the official method for testing Liquor Antisepticus N. F. by the National Formulary (8th Edition, 1946). For the reasons given above, there is general agreement that the F. D. A. methods of testing antiseptics and disinfectants are not only standard, but official as well.

It will not be necessary to describe these methods in detail, but it may be of interest to examine the background of the various tests and to discuss their interpretation. This subject is as important now as it was in the past, since the basis of these tests must be thoroughly understood before they may be interpreted properly. Although the F. D. A. methods of testing have been standard for 22 years and official for 17 years, there are many who are not acquainted with the background and the reasons for using these specified procedures.

Regulation and control of antiseptics was initiated by the Bureau of Chemistry, U. S. Department of Agriculture, in 1926. Although disinfectants had been controlled by the Insecticide Division since 1915, it was not until ten years later that antiseptics were regulated, first by the Drug Control Division of the Bureau of Chemistry, later by the Food and Drug Administration. Since there were no suitable methods at that time, it was necessary to develop laboratory methods of tests, methods which would reflect the value of antiseptics, when applied under practical conditions of use.

In cooperation with L. P. Shippen, the author devised and established the specifications which later became known as the F. D. A. methods for the testing of antiseptics. It was, of course, necessary to base these methods on known values of proved merit, since the procedures would be used in the regulation and control of antiseptics in interstate commerce. For this purpose it was necessary to establish standards of comparison.

Liquid Antiseptics

Since 2.0% phenol had for many years proved effective as an antiseptic, the test for liquid antiseptics was designed to fit this germicide of proved merit. Also, since antiseptics are used to prevent wound infections, it was logical to require them to kill the most

common cause of such infections, namely *Staphylococcus aureus*. Then, as a factor of safety, the test was so designed that products were required to kill large numbers of this infectious organism, far more than would be present in wounds, abrasions, cuts, etc.

The methods of test, devised for the various kinds of antiseptics, made use of a strain of *Staphylococcus aureus* (No. 209) which was representative of the most resistant staphylococcus freshly isolated from suppurative infections (7). The formula of the media employed was designed to maintain this resistance. The effect of peptone on the resistance of the test organism was emphasized and the particular brand used was specified (8). The details of the method of test were set forth (3) and these methods have become known officially as the U. S. Food and Drug Administration Methods (6).

Although there are only approximately 40 bacteria per square centimeter of dirty skin, and only about 10 to 15 million bacteria per ml. of saliva, the official F. D. A. test for liquid antiseptics requires the use of 350 million of the most resistant of all skin and mouth bacteria. This excessive number of staphylococci must, according to official Circular 198, be killed by the antiseptic wthin 5 minutes. This is for the purpose of supplying an adequate margin of safety.

Another reason for using this large number of pathogenic organisms is that known antiseptics of proved merit are able to pass the test. Since phenol had already been recognized as a satisfactory and suitable standard germicide for comparing disinfectants (9) (10) (11) (12) it was quite naturally employed as a standard for antiseptics. The reason for selecting 2.0% was that first 2.5% and later 2.0% phenol were found to be effective antiseptics in clinical practice.

Despite the fact that 2.0% phenol was proved clinically effective, it will not pass the F. D. A. test when diluted with an equal part of water.

Since 2% phenol is so generally recognized as an effective antiseptic, it serves well as a standard of comparison. However, antiseptics are not compared to phenol as in the phenol coefficient of disinfectants; (13) (14) they are merely *germicidally equivalent* to 2% phenol. The F. D. A. test classifies antiseptics as those that are at least equal to 2% phenol and those that are not. In other words, products which pass this test may be considered antiseptic.

A number of well established antiseptics were able to pass this new test, some of them official preparations of known antiseptic merit. It thus became apparent that the F. D. A. test effectively reflected efficient antiseptic action and for this reason it proved to be a satisfactory test for the purpose.

The F. D. A. method, first published in 1926 (3) (11) (15) and established as an official test in 1931, (6), is still found satisfactory for the purpose for which it was designed. In fact, no more satisfactory method of test has so far been proposed. As a result, the F. D. A. method continues as the standard test for liquid antiseptics in this country.

It is of interest to note that the only criticism made of this test when it was first announced was that it is too severe. There was considerable objection to the test, but only because it seemed unduly stringent. The reason for this is that the safety factor is inherent in the test. It does not require the use of a factor for determining proper dilution for use, as is the case with tests of disinfectants (16). This being the case, the necessity for severity of the test is understandable.

The general use of this test made it possible to standardize antiseptics on a sound basis (17). As a result, the control of antiseptics has been made more effective. Also, this effective control assures the professions and the public of antiseptics of known germicidal efficiency. This has repeatedly been confirmed by comparative studies by laboratory and practical tests (18) as well as by clinical experience in actual use of antiseptics passing the standard official test.

More recently, criticisms have been made of the F. D. A. method of testing liquid antiseptics because the test does not "simulate practical conditions of use." The test was never intended to duplicate clinical conditions for the simple reason that no single test can possibly simulate all the clinical conditions for which antiseptics are used. Since it is impossible to simulate all conditions, the best that can be done is to use a test that has been proved by actual clinical experience. This is exactly the situation in the case of the F. D. A. method, since this test is based on known values, as determined by practical and clinical experiences.

In spite of this, efforts were made to devise practical tests which were supposed to simulate actual clinical conditions. In one such

test suggested over ten years ago, the infected skin of laboratory animals was treated with germicide, and then the skin was cultured in nutrient media and inoculated into animals. This did not prove to be a satisfactory procedure, therefore it was not widely employed. There are too many variables which cannot be controlled and there is no factor of safety in the test. The test does not simulate practical use of antiseptics in wounds or first aid. If, for no other reason, the variability of results obtained by this method alone would preclude its use as an accepted procedure for testing antiseptics.

At about the same time, effort was made in another test to prevent infection by use of germicides on skin that had been artificially contaminated with infectious organisms, then treated with the antiseptic to be tested. The treated areas were scraped with a scalpel until serum exuded from the resulting abrasion. After removing and culturing the scraped skin, the animals were observed for infections of the treated areas. The control areas, to which no germicide was applied, did not become infected with any degree of uniformity. In the absence of sharp foreign bodies, it is difficult to obtain infections under these conditions, therefore comparisons were not entirely valid.

More Recently Proposed Methods for Testing Antiseptics

Within the past ten years a number of new methods for studying and testing antiseptics have been proposed. This review will present the methods in chronological order rather than grouping them according to the nature of the test.

An *in vivo* method was suggested in which tissue of living animals is first infected with a pathogenic organism, then treated by the application of antiseptic (*21*). However, it was impossible to duplicate conditions of use and results were so variable that the test could not be considered as a standard procedure. Also, there is no factor of safety in the test. For these reasons the test has not received wide recognition.

One of the most promising of the proposed methods was an *in vivo* test recommended as an "infection prevention test" for evaluating skin antiseptics (*22*). This test makes use of white mice. Test organisms are applied to the tip of the tail, after which the tail is dipped into the antiseptic, and allowed to act for 2 minutes. Then

the tip of the tail is amputated and inserted into the peritoneal cavity of the same mouse. Much can be said in support of this test for the purpose indicated, that is, for determining the value of antiseptics applied to the unbroken skin. The results obtained are consistent and are not subject to the previously mentioned variables in other *in vivo* tests.

The principal objection to this test, however, is the nature of the test organisms used. In order that this test be effective, organisms pathogenic for mice must be employed. For this purpose, one of the organisms used is the pneumococcus because white mice are especially susceptible to intraperitoneal injection of this organism. Actually, however, the pneumococus is not normally present on the skin, is not responsible for skin diseases, and is not a factor in post-operative infections. For this reason, results obtained with this test organism could not be interpreted in terms of practical value for human use. Also, the pneumococcus is a very weak organism and is easily killed by antiseptics. In other words, a skin antiseptic which would pass this test by killing the pneumococci would not necessarily kill the staphylococci which are normal inhabitants of the skin and the most common cause of wound infections. And in addition the test does not contain a safety factor.

According to results obtained by this test, tincture of iodine 2% was the only antiseptic that proved completely effective. While tincture of iodine is recognized as an effective skin antiseptic, satisfactory clinical results may be obtained with a number of other antiseptics. Therefore, results obtained by this test are not fully supported by clinical experience.

The only other pathogenic organism used in this "infection prevention test" is a hemolytic streptococcus. Here again, this organism is not a constant inhabitant of the skin nor is it a common cause of post-operative infection. Also, hemolytic streptococci are much weaker than staphylococci, and therefore more easily killed by antiseptics. In fact, both pneumococci and hemolytic streptococci are only about half as resistant to germicides as *Staphylococcus aureus*. Here again there is no assurance that the killing of hemolytic streptococci will mean that staphylococci will be affected. There is no margin of safety in the test, because a weak test organism is employed.

Since this is the most reasonable *in vivo* test suggested so far, it is important to review the above objections to the test. As compared with the F. D. A. test, this test is definitely weak because weaker test organisms are used. It is weaker also in that far fewer organisms are employed in the test. In addition, no provision is made for maintaining, or even assuring, the normal resistance of the test organisms. There are no detailed specifications for the media employed for culturing the test organisms. It would be impossible to duplicate results with different strains of these test organisms, or even with the same strains in different laboratories. In other words, there are no detailed specifications as to the culture, and no precise standardization of the procedure. It must be borne in mind also that the use of this test is limited to skin antiseptics and is not intended as a test for general antiseptics.

The F. D. A. test, on the other hand, is designed for testing antiseptics for general use. Also, the specifications for the details of the F. D. A. test are such that it is easily standardized. As a result, consistent results are obtained in different laboratories throughout the country. It is a severe test, using the most resistant of nonsporing organisms, the one causing the greatest number of wound infections. Also, and this is extremely important, the factor of safety is inherent in the test itself. Thus it becomes apparent that the F. D. A. method is far superior, in every respect, to the above described "infection prevention test."

Another practical test proposed is one in which reduction of numbers of bacteria on the skin are checked by simple agar plate counts, that is, bacteria remaining on the skin after application of antiseptics are counted (23) (24). While this method is applicable to the study of bacterial reduction on the skin of an individual, it cannot be applied to studies of antiseptic action generally, such as in first aid, oral application, etc. Also, test results obtained on different individuals vary considerably. Therefore the effectiveness of antiseptics applied to the skin cannot be estimated from results obtained on any one subject. In addition, the test is quite time-consuming and involves an inordinate amount of work, considering the value of the information obtained.

While the information obtained from this test is interesting, it cannot be considered a suitable method for testing general antiseptics. It is specifically designed for and is limited to the testing of

skin antiseptics. When used for this purpose, the information obtained is useful only for determining the efficacy of antiseptics applied to normal, unbroken skin. Antiseptic soaps may be tested by this procedure, but here again, the results cannot be interpreted as applicable to all individuals. Because of the variability of results obtained on differing subjects and because of the time-consuming nature of the procedure, this method is not widely used.

There have, of course, been many suggestions that the laboratory testing of antiseptics simulate practical conditions of use. Such tests should, if possible, also indicate the degree of toxicity of antiseptics for human and animal tissue. Efforts have been made to devise tests combining these two features.

Among the first such methods presented was one which made use of a "toxicity index," obtained by exposing both embryonic chicken heart tissue and a culture of *Staphylococcus albus* to an antiseptic, in order to compare inhibition of tissue growth with germicidal action (25). While this is a very interesting method, its use is limited to research. It is an *in vitro* method that does not in any sense simulate practical conditions of use of antiseptics.

In the first place, embryonic chicken heart muscle is not comparable to human or animal tissue. No actual relationship exists between this tissue and skin, mucous membranes, or areas surrounding wounds. Toxicity to embryonic chicken heart muscle does not mean equivalent toxicity to epithelial tissue, for example. In other words, this test is not in any sense a practical one.

Furthermore, results obtained by this "toxicity index test" do not properly classify antiseptics in terms of usefulness under clinical conditions. For example, it was necessary to use a 1-1800 dilution of iodine in order to arrive at a "toxicity index" for this antiseptic. However, in practice, 2% tincture of iodine is used as an antiseptic. Actually there is no clinical relationship between the toxicity to tissue of a 1-1800 dilution of iodine and of a 2% or 7% tincture. No surgeon would rely upon a 1-1800 dilution of iodine. He would use the standard, official tincture of iodine, either the mild (2%) or the strong (7%). Therefore, what practical interest could possibly pertain to the lack of toxicity of 1-1800 aqueous iodine when concentrations of 2% and 7% in alcohol are the ones used? There is no way in which results of this "toxicity index test" can be interpreted in terms of practical value.

For example, under the conditions of this test it has been shown that Mercurochrome has a "toxicity index" 2,620 times greater than iodine, yet it cannot be shown that 2% Mercurochrome is 2,620 times more toxic to tissue than 2% tincture of iodine as the ordinary use of the terms indicates. As a matter of fact, if Mercurochrome was actually 2,620 times more toxic than iodine or even 6 times more toxic, it could not be used safely on human or animal tissue. Actually it is only 6 times more "toxic" to chicken heart muscle tissue ($10{,}500 \div 1{,}800 = 6$) and not 2,620 as the index states. Because results show that the "toxicity index" of Mercurochrome is 262 and that of iodine is only 0.09, such an "index" is misleading and cannot be interpreted in terms of practical value.

The following are valid objections to this "toxicity index test" procedure: (1) embryonic chicken heart muscle does not represent human or animal skin, mucous membrane, or tissue exposed in wounds; (2) the test requires exposure of the tissue to antiseptic for 48 hours, whereas in actual use, antiseptics are ordinarily applied for much shorter time periods—in some instances only minutes; (3) concentrations of the antiseptics used in this test are many times weaker than concentrations used in practice; and (4) the conditions of the test do not in any sense simulate the clinical conditions under which antiseptics are used. Therefore, the results obtained by this test cannot be interpreted in terms of practical value.

Another suggested method of evaluating antiseptics by comparing germicidal activity with toxicity is one in which leucocytes and skin are employed as tissue (26). This method, published at about the same time as the above "toxicity index" method, has many desirable features and approximates clinical conditions more closely than many other laboratory test procedures. This method is a combination of (1) germicidal tests by the F. D. A. method for liquid antiseptics, (2) diffusibility tests through paralodion sacs, (3) a toxicity test against leucocytes, and (4) tests for intradermal toxicity.

The object of this test is to determine which antiseptics are best suited for use in wounds, cuts, abrasions, and cavities—in other words, for surgical use. The ideal antiseptic for surgical use should possess positive germicidal activity against pus-forming organisms, should be active in the presence of serum and blood, should be diffusible—that is, should penetrate into body tissue—should be relatively non-toxic in the dilutions employed, and should be inexpensive.

The author of this test has considered all of these factors in his study. His methods and interpretation of results seem reasonable, logical, and scientific.

In this test, the standard Food and Drug Administration Method for Liquid Antiseptics is used both with and without blood serum, for determining germicidal activity. Diffusibility tests are made with paralodion sacs, the antiseptics being placed inside the sacs and the dialysate tested for germicidal activity.

The test for toxicity is rather unique. White blood cells (leucocytes) constitute an important defensive factor against infection. When they are killed by a germicide, they are prevented from accomplishing their normal function of engulfing and killing infectious bacteria. This test is one which shows whether or not white blood cells are killed by the germicide used, in the dilution usually employed.

In some respects this test is superior to the first "toxicity index test" described. In the first place, human skin is employed instead of embryonic chicken heart tissue. Also, exposure times to the tissue and to the test bacteria (*Staphylococcus aureus*) are comparable, not widely different as in the first "toxicity index test," in which tissue is exposed to test antiseptics for 48 hours and to *Staphylococcus aureus* for only 10 minutes. The tests for penetration through blood serum and the tests for diffusibility through paralodion are of much importance, and results obtained are of significance in terms of practical value.

The only serious objection to this method is the use of leucocytes as the medium of test for tissue toxicity. Leucocytes do not represent body tissue generally and the effect of antiseptics on leucocytes cannot be interpreted as effect on those human and animal tissues to which antiseptics are ordinarily applied. Also, leucocytes are single cells that may be easily surrounded by liquid antiseptics, whereas cells of other tissues, such as skin, mucous membrane, and muscle, are not so easily attacked by liquids. Certainly no "toxicity index" or "toxicity test" of any kind should be based only upon effect on leucocytes.

Because leucocytes are a part of the body's defense mechanism, it has been suggested that they should not be destroyed by an antiseptic, since by doing so this defense factor would be eliminated. This is not actually true, since there is practically an inexhaustible

supply of leucocytes in the body. If leucocytes are destroyed in a wound, for example, more are supplied to the site of injury. Therefore, it is of no practical importance whatever if those present in the area are destroyed at any one time, since more are continually supplied to take their place. In other words, it is far more important to kill the invading infectious bacteria, even if those leucocytes present at the time are destroyed. Therefore, locally present leucocytes need not be considered in the practical use of antiseptics.

A few years later a second method for computing a "toxicity index" by the use of leucocytes was suggested (*27*). We will discuss this test in some detail because, according to the authors, "In the development of the proposed method an attempt has been made to approach as nearly as possible the conditions under which antiseptics are used." The method proposed resembles the one just discussed, in that leucocytes are used for testing toxicity. But it fails to meet the authors' promise to simulate conditions under which antiseptics are actually used. In fact, it does not approach this ideal as closely as the first leucocyte method discussed, since it makes no provision for testing for toxicity against such tissue as skin, or does it test for diffusibility and penetration of tissue by antiseptics.

Under the conditions of this test, the leucocytes and the test organism *Staphylococcus aureus* are exposed to the antiseptic for 30 minutes. Observations are made as to the ability of the leucocytes to phagocytize the staphylococci. The "toxicity index" is determined by dividing the highest dilution "toxic" for the leucocytes by the highest dilution killing the test organism. If presence of the antiseptic inhibits phagocytosis in a dilution greater than the germicidal dilution, the "toxicity index" is above 1. If the dilution inhibiting the leucocytes is more concentrated than that killing the test organism, the "toxicity index" is less than 1. In this connection, it is of interest to note that inhibition of phagocytosis is designated as "toxicity to tissue" and not simply as inhibition of leucocytic activity. Also, the figure obtained by dividing the dilution inhibiting phagocytosis by the dilution killing *Staphylococcus aureus* is designated as the "toxicity index."

As explained earlier, although leucocytes are tissue, leucocytes do *not* represent those body tissues to which antiseptics are ordinarily applied. They do not possess a resistance to antiseptics and other drugs that is comparable to such resistance by epithelium,

mucous membrane, etc. Therefore, inhibition of leucocytes cannot be considered as "tissue" toxicity. The term has been misused.

Apparently, the object of this test is to compare germicidal activity with the ability of the antiseptic to injure body tissue. It is indicated that antiseptics which "inhibit" the activity of leucocytes in a dilution in which they are germicidal should not be used—or at least that they might be harmful. The authors state that harm resulting from use of such germicides is two fold: (1) leucocytes would be prevented from killing bacteria, and (2) the antiseptic would actually injure body cells. In this paper the first premise is the one emphasized, but the second is implied.

In the first place, results reported do not show whether or not the leucocytes themselves were actually destroyed or even injured. All that is shown is that phagocytosis has been prevented by the antiseptic. It is not shown why the leucocytes were rendered inactive. Since leucocytes can be restrained from exercising their phagocytic function without a toxic effect being exerted—for example, by interference with humoral elements of the blood, such as opsonins—the results of this test cannot properly be designated as a "toxicity index."

In another paper (28) it has been shown that inhibition of leucocytes by antiseptics is due to destruction of opsonic complement, or opsonins. Since opsonins, normally present in the blood, are necessary in order for leucocytes to kill bacteria, anything which destroys this substance will prevent phagocytosis. Opsonins are nonliving, therefore their destruction is not due to toxic action. If chemical alteration (destruction) of opsonins is the reason for inhibition of phagocytosis in this test, the results obtained could not possibly indicate "toxicity" of the antiseptics.

This test has been applied to oral antiseptics, despite the fact that neither opsonins nor leucocytes in any quantity are present in the mouth and throat. Opsonins are normally present in blood only. Since blood is not present in the normal mouth, any leucocytes which may possibly be present in the oral cavity cannot phagocytize bacteria for the simple reason that activating opsonins are not present. This fact must have been recognized by the authors of the method because in testing oral antiseptics they found it necessary to add normal serum to a mixture of leucocytes in saliva in order to produce phagocytosis.

Because the few leucocytes that may be present in the oral cavity are inert, or inactive from the standpoint of phagocytosis because opsonin is not present to activate them, it is of no importance in the testing of oral antiseptics that the antiseptic may destroy opsonin. Opsonin is not present in the mouth and throat anyway. It would not even be of importance if an oral antiseptic injured or destroyed such leucocytes as might be present, for they are not capable of killing bacteria in the mouth and throat.

There is no evidence that the few leucocytes at times observed in the oral cavity actually exert any phagocytic activity whatever while in the mouth and throat. Indications are that they are merely cells that have become devitalized after leaving the capillaries. Such leucocytes as have been observed containing bacteria probably engulfed the organisms before entering the oral cavity.

Attempts to count the number of leucocytes present in the mouth meet with little success because they are so few in number (almost none) and stain so poorly. Since normal leucocytes stain readily and are easy to see when stained, the fact that leucocytes found in the mouth are difficult to see indicates that they have lost their vitality—that is, they are no longer viable or capable of exerting their normal function.

Even supposing leucocytes in the mouth and throat were normal and present in large numbers, and supposing opsonins from the blood were present so that they could function, it still would be of no importance if the leucocytes were killed by an oral antiseptic. Leucocytes are continually being formed in the body and these quickly take the place of any that might be killed by the antiseptic. In fact, stimulation of the tissue by chemical and other means causes an increase in the production of leucocytes.

Results obtained by this test are called "toxicity indices." In other words, inhibition of phagocytosis is considered a toxic action against a body tissue. White blood cells are used as "tissue," which would indicate that they are supposed to be representative of tissue. Blood cells are not a representative tissue, therefore a test using leucocytes as the indicator of tissue toxicity cannot be a true index of the effect of drug and chemical agents on body cells generally. Leucocytes are more easily affected by antiseptics, are more easily killed, than are cells of the skin and mucous membrane.

In spite of the fact that viable leucocytes are not present in the oral cavity, this method has been applied for testing the "toxicity" of oral antiseptics (29). A total of 87 commercial oral antiseptics were submitted to the test. It is of interest to note that a dilution of 1-5 of the antiseptic is required by the test (0.1 ml. antiseptic, 0.2 ml. antigen, 0.2 ml. human blood). This dilution was made in spite of the fact that most of the oral antiseptics tested specify on their labels that they are to be used undiluted. It was therefore unreasonable and unfair that they be tested for germicidal activity in a dilution five times greater than is recommended on the labels.

Further, this "tissue toxicity test" was done in the presence of 40% blood. Since there is no blood in the normal mouth or even in sore throat, oral antiseptics need not be effective in the presence of blood. Such products should not be tested in 40% blood since they are not intended for use in the presence of blood.

Not only that, but in this test the antiseptic is exposed to leucocytes (and bacteria) for 30 minutes in a closed glass tube. This does not even simulate the conditions under which such products are used. Oral antiseptics are usually held in the mouth for only about 30 seconds. Although a small residue remains in the mouth for several minutes afterward, this is by no means comparable to continuous contact of germicide and leucocytes for 30 minutes in a closed tube.

This "toxicity index" is not even of value in testing antiseptics recommended for use in cuts and wounds. The reason is that leucocytes act only in tissue. If bacteria and leucocytes are killed on the surface of the cut or wound, no harm will result, since active leucocytes present in the capillaries will continue to function. Also, new phagocytes and opsonic complement are continuously carried to the site of injury, for the blood contains a practically inexhaustible supply. Many thousands of times more leucocytes and opsonic complement are removed when blood is taken for a transfusion than could possibly be destroyed by the local application of an antiseptic to a cut or wound, yet no harm results.

Physicans use antiseptics as wet dressings on wounds with no harmful effect. Under these circumstances the leucocytes present in the wound are either killed or inhibited, but the wound does not become infected. Physicians consider it far more important to kill the infectious bacteria than to spare the locally present leucocytes.

The only value this so-called "toxicity index test" might have would be for antiseptics used for intravenous medication. Even for such use, practical experience is more valuable. For instance, Mercurochrome, which has the extremely high "toxicity index" of 32.75 by this test, can be injected into the blood stream without harmful effect. So it may be seen that even in testing antiseptics for intravenous use, this index has no valid clinical significance.

At the other extreme is iodine, which, according to this test, has a "toxicity index" of only 0.79. But in order to secure this low figure, Lugol's solution had to be diluted 1-60 and tincture of iodine 1-84, giving a final concentration of 1-600 of iodine. It stands to reason that no physician or surgeon would dare to use such dilute solutions of iodine for antiseptics. On the other hand, the iodine solution he *does* use, that is Lugol's, or 2% and 7% tincture of iodine, all kill leucocytes—further proof that this so-called "toxicity index" is without practical value.

The authors of this test report that iodine is the only antiseptic showing a "toxicity index" of less than 1, that is, iodine is the only antiseptic that is less toxic to leucocytes than it is to *Staphylococcus aureus.* However, in order to be able to assign a "toxicity index" of less than 1 to iodine, they were forced to use 1-600 test solutions—to dilute the official iodine solutions 1-60 and 1-84. The test's authors failed to state, however, that there is no clinical evidence whatever that 1-60 Lugol's solution and 1-84 dilution of 2% tincture of iodine are germicidally effective. The only clinical evidence we have of the value of iodine solution as an antiseptic is the use of *undiluted* Lugol's and tincture of iodine.

For all of the reasons presented, it becomes apparent that the "toxicity index," as determined against leucocytes, is without practical significance. The indices can be of only theoretic interest. The test itself should be reserved for use only in chemo-therapeutic research. It should not be employed as a test for antiseptics.

In a still later paper (30) this test was modified to show actual destruction of leucocytes, instead of mere inhibition. The results are reported as "toxic activity," using human blood as the test tissue. The irreversible loss of phagocytic action after 10-minute exposure to antiseptic is taken as an indication of cell death, and the results obtained are designated as "toxicity of antiseptics" despite the fact that leucocytes are not representative of body cells generally.

One of the principal difficulties in laboratory testing of mercurial germicides has been the high bacteriostatic activity of such compounds (31) (32). The difficulty has been largely overcome by use of thioglycollate as the subculture medium (33). Use of this medium in the testing of mercurial antiseptics permits determination of the true germicidal properties of mercurials and eliminates much of the confusion caused by bacteriostatic activity in the subculture media itself. Thioglycollate inactivates mercurials carried over into subculture media, thus surviving organisms can grow. This is the same medium used to test biologicals for sterility (34) (35).

The thioglycollate method of *in vitro* testing of antiseptics has received general use during the past few years. It has been found so satisfactory that it often replaces other *in vitro* tests. Accurate testing of antiseptics, especially the mercurials and quaternary ammonium compounds, has been greatly facilitated by use of this medium.

Another *in vivo* test, which improved previous methods, uses the skin of white mice and *Streptococcus hemolyticus*, a test organism that is capable of causing peritonitis (36). The streptococcus culture is applied to the slightly abraded skin of white mice; the germicide is then applied to the area for a specified time period. Then pieces of the treated skin are inserted into the peritoneal cavity of the mouse. Virulence of the test organism is enhanced by animal passage before use. The germicide is allowed to act for three minutes before bits of skin are inserted into the peritoneal cavity. Failure to develop infection is proof that the test organism was killed by the antiseptic.

By this test, tincture of iodine gives the best protection against infection. Other antiseptics tested are much less effective, in varying degrees. The only objection to this method is that the test organism *Streptococcus hemolyticus* is much more easily killed by germicides than is *Staphylococcus aureus*, the most common inhabitant of the skin. So for this reason the test does not provide the factor of safety which is so important in any test for the germicidal activity of antiseptics.

One of the newer *in vivo* methods for testing antiseptics uses as tissue the chorio-allantoic membranes of eggs containing live embryos, and *Staphylococcus aureus* as the test organism (37). The chorio-allantoic membranes of eggs containing embryos 12 days old are inoculated with a small quantity (0.02 ml.) of a dilute (1-10)

suspension of broth culture of *Staphylococcus aureus.* Eighteen hours later and on each of the 5 following days, 0.2 ml. of the test antiseptic is dropped on the membrane. The day after the last treatment, the membrane is stroked with a cotton swab, then streaked on an agar plate. After incubation, the plates are counted for numbers of surviving organisms.

By this method penicillin and the cationic detergents tested were found to be effective in killing the test organism, whereas iodine, phenol, and the organic mercurials employed were ineffective. It is inferred from these results that penicillin and the cationic detergents should possess therapeutic value under clinical conditions of use, whereas iodine, phenol, and the organic mercurials could *not* be clinically effective.

While this method of test simulates wound conditions more closely than do the other *in vivo* or *in vitro* tests previously suggested, it still fails to satisfy the conditions required of a satisfactory test procedure for antiseptics. The chorio-allantoic membrane of a fertile egg does not simulate such human or animal tissues as skin or mucous membranes. Also, the amount of culture used in the test is too small to allow for a factor of safety. But still more important, the antiseptics tested were applied in far greater dilution than is recommended for use in clinical practice. For example, iodine was used in dilutions of 1-2,500, 1-5,000, and 1-10,000, whereas 2% and 7% iodine are used in clinical practice. No physician or surgeon would use such weak solutions of iodine in first aid or in wounds. A 1-50 solution of iodine is the weakest dilution acceptable for general use. And finally, there is no provision in the method to prevent bacteriostatic activity of test antiseptics when the agar plates are swabbed from the treated membranes.

It is interesting to note that the antiseptics giving the best results by this test are those that are highly bacteriostatic against the test organism, *Stapyhlococcus aureus.* It could well be, and probably is, that their bacteriostatic effect on the agar plate accounts for the favorable results reported. On the other hand, iodine, phenol, and Azochloramide, in the dilutions used, are not so highly bacteriostatic under the conditions of this test. Also, Metaphen and Merthiolate could hardly be expected to exert their effect in the dilutions used, namely, 1-5,000 to 1-30,000, when they are recommended for clinical use in 1-500 to 1-1,000 dilutions.

Here again it is necessary to take a practical view of the problem. While it may be desirable to use a dilution of antiseptic that would not injure such delicate tissue as the chorio-allantoic membrane of fertile eggs, in human and animal practice it is far more important to kill the infective organisms. Infection must be controlled even if the antiseptic does destroy some of the tissue cells. In actual clinical practice, the antiseptics are used in concentrations that kill infective organisms, such as 1-50 phenol, 1-50 iodine, 1-500 Metaphen, 1-1,000 Merthiolate, etc. Even though these concentrations of the antiseptics do destroy chorio-allantoic membrane, still they prevent infection by killing the invading bacteria, and at the same time they cause no serious damage to the human or animal tissues on which they are used.

It is, of course, desirable and even necessary to conduct a variety of tests on antiseptics in order to evaluate their possibilities for clinical use. This is the general consensus of bacteriologists and physicians actively interested in this field. Tests should be conducted to determine their various properties under a variety of conditions. One single test is not sufficient for this purpose and, in fact, it is not expected that any one test will supply all of the information necessary to evaluate the properties of an antiseptic.

Recently a very excellent suggestion has been made for the profile evaluation of germicides (38). A combination of well-established tests is recommended for the purpose. Use of these tests is specified for determining the following properties of antiseptics: (1) the highest dilution of germicide capable of killing *Staphylococcus aureus;* (2) extent of bacteriostasis; (3) influence of organic matter; (4) speed of action; (5) penetrability; and (6) toxicity.

For this purpose the following tests are employed: (1) modification of the F. D. A. test for determining killing dilution against *Staphylococcus aureus* (using standardized suspension); (2) bacteriostatic tests in 10 ml. and 100 ml. F. D. A. broth and thioglycollate medium; (3) similar tests in the presence of 10% horse serum; (4) tests for killing, in time periods of from 1 to 10 minutes; (5) penetration tests, using the F. D. A. agar cup plate method, both with and without 10% horse serum; and (6) toxicity tests on embryonic chicken heart tissue.

Studies of this character give considerable information relative to the properties of antiseptics. In fact, a combination of these tests

will give information from which the practical value of an antiseptic may be predicted, or at least they will reflect its potentialities. Furthermore, the methods employed are all standard procedures that have been used separately for some years. It is now proposed that all of these tests be conducted as a panel. Since each test shows certain specific properties of antiseptics, collective results give a correct and useful profile of the antiseptics tested.

Just a few months ago a serious effort to determine the germicidal spectrum of various kinds of antiseptics against a variety of test organisms was reported (39). The method employed was a variation of the phenol coefficient type of test procedure, using a large number of representative pathogenic organisms. The purpose of the study was to determine the germicidal activity of representative germicides, including mercury compounds, phenol, cresol, hydrogen peroxide, alkali, acid, alcohol, silver nitrate, iodine, and quaternary ammonium compound. Fourteen micro-organisms, of pathogenic or potentially pathogenic species, were used as test organisms. Cultures were grown in 2% tryticase and after exposure to various dilutions of antiseptics were subcultured into U. S. P. liquid thioglycollate medium.

Results of these tests showed the wide variation in resistance of different species of the same test organisms and also between the various classes of bacteria. It was evident from results obtained that the resistance of test organisms must be determined before they are employed in laboratory tests on germicides. Also, it was shown that it is desirable to use a variety of such test cultures in order to obtain a full bactericidal spectrum. This test should, by all means, be included in studies of germicidal activity of antiseptics.

One of the most interesting recent developments in laboratory testing of antiseptics is the current controversy on the germicidal activity of organic mercurials. Several important factors which require careful analysis are involved in this matter.

It has been generally known for many years that mercurial antiseptics are more highly bacteriostatic than germicidal. This has been proved repeatedy by laboratory and practical tests, both *in vitro* and *in vivo*. Therefore, the information given in recent publications on the subject is not surprising. Interpretation of the new data presented, however, must be considered in the light of present

knowledge of the whole matter of testing antiseptics and also in terms of practical application.

Before discussing the controversial aspects of laboratory testing of organic mercurials, it is important to keep in mind the definition of the word "antiseptic," for the accepted definition of the term covers both bactericidal and bacteriostatic properties. It is immaterial which of these properties an antiseptic possesses, provided it renders infectious organisms innocuous when used as directed—that is, if it acts "against infection" under clinical condition of use.

Recently a thorough restudy of the antiseptic properties of certain organic mercurials was made (40). The objectives of the study were to determine "(1) the effect of some of the organic mercurial compounds on an organism pathogenic for man and mouse and (2) whether the growth of the test organism in thioglycollate medium parallels infectivity for the animal body."

The organism used in this study was *Streptococcus pyogenes* (Strain C 203 M) which gives a wide zone of hemolysis on blood agar and is pathogenic for man and some laboratory animals. White mice were regularly killed with this organism by intraperitoneal injection of 1 ml. of a 1:10,000,000 dilution of a twenty-four hour culture. This lethal dose gave only four colonies when grown in blood extract agar.

The organic mercurials employed in the studies were Mercurochrome, Merthiolate, and Metaphen. The technique employed in the test procedure differed in some essential features from present standard methods. The medication temperature was 20°C. instead of the standard 37°C. temperature used for testing of antiseptics. Subcultures were made by inoculating 0.5 ml. of the antiseptic-culture mixture into subculture media, instead of 1 loopful as in the standard procedure. These two modifications alone render this test method much more severe than the already severe F. D. A. test for liquid antiseptics. Also, the number of lethal test doses employed, namely 5,000,000, is much higher than is required for simulating practical conditions of use. In other words, the test requires the killing of 5,000,000 lethal doses of *S. hemolyticus* at 20°C. This is loading the test against the antiseptics far beyond the point necessary to test practical values.

The results of these tests, as might be expected, appear to demonstrate that the organic mercurials employed were weak germi-

cides. These results, however, cannot be interpreted in terms of practical value for the simple reason that the conditions of the laboratory test are abnormally and unjustifiably severe. This is true for the following reasons:

1. The numbers of streptococci present under clinical conditions of use are far fewer than are used in the test (many times less than the 5,000,000 lethal doses employed).
2. The temperature of the skin is 33°C, not 20°C and the temperature of tissue surrounding wounds is near 37°C, not 20°C.
3. The amount of antiseptic-culture mixture inoculated into subculture tubes is 25 times greater than that employed in the severe standard methods in universal use.
4. Since this unduly severe test does not simulate clinical conditions, the results obtained cannot be interpreted in terms of practical value.

The conclusions the authors draw from their results are untenable as a clinical evaluation of these mercurial antiseptics. This is clearly emphasized in criticisms of the paper, published six months later (41) (42). It was shown that when the number of test organisms is reduced to that approaching practical clinical conditions, both Mercurochrome and Merthiolate are demonstrated to be actively antibacterial in effect—even under the conditions of a test procedure not suitable for testing antiseptics, that is, at 20°C medication temperature. In fact, more than 80% of the test animals survived excessive lethal doses of test organisms that had been exposed to the antiseptics at 20°C for 10 minutes. When the intravenous method of inoculating the animals was used instead of the intraperitoneal route, none of the animals were killed by massive lethal doses after exposure to the antiseptics. Also, these excellent results were obtained with a concentration of antiseptic only one-half that marketed to the professions and the laity. Even when 100,000 lethal doses of the streptococci were used, these antiseptics proved germicidal under the conditions of this test method. These re-evaluations of the original tests prove that effectiveness of the

mercurial antiseptics tested depends upon time, temperature, and the number of organisms used.

In this connection it is well to emphasize the fact that when used clinically, antiseptics continue to act for varying periods of time. In the case of organic mercurials, this is usually an extended period, certainly far longer than the time periods used in these tests. Some germicides act slowly but, since they are also bacteriostatic during the period of exposure, this is no real disadvantage.

On the other hand, some antiseptics kill bacteria quickly, but are not highly bacteriostatic. Liquor Antisepticus N. F. and similar germicides belong to this class. In fact, one such antiseptic has been shown to kill hemolytic streptococci within 15 seconds when 0.5 or even 1.0 cc. of undiluted broth culture of these organisms is exposed to 5.0 cc. of the antiseptic at 37°C. Such quick-acting germicides, however, are not necessarily better than the slower-acting organic mercurials. They act "against infection" in their own way, and they are both effectively antiseptic under clinical conditions of use.

Of course, the final criteria for an antiseptic is effectiveness and usefulness in clinical practice. The vast amount of published data supporting the clinical effectiveness of organic mercurials constitutes sufficient evidence as to their value as antiseptics for general use. This is an extremely important factor that should be given first consideration in any study of the antiseptic properties not only of organic mercurials, but of all other antiseptics as well.

Fungicides and Fungistats

A review of recent developments in the field of antiseptics would not be complete without some reference to a significant improvement in laboratory methods for testing fungicides and fungistats used in the treatement of fungus infections. Although such fungicides are not usually classed as antiseptics, they are drugs which are used on the human and animal body for the purpose of acting "against infection." This being the case, they fit the definition of antiseptics, so should be classified similarly. Also, as most fungicides recommended for human use are also employed in the prevention of bacterial infections, they may well be considered in this report.

Fungicides are subject to control by the Food and Drug Administration as antiseptics and they are considered antiseptics by the

professions and the laity. However, special methods of testing are required for fungicides—the higher fungi are employed as test organisms instead of bacteria.

The most important development in this field during the past ten years is the laboratory method proposed for testing fungicides used in the treatment of epidermophytosis (43). In this method the five fungi most commonly found in athlete's foot are used as test organisms. Methods for determining both fungicidal and fungistatic activity are employed in the tests. The methods are severe and to some degree simulate clinical conditions of use. Fungicides and fungistatic products passing these tests have also been found clinically effective under practical use conditions.

Within the past two years the fungistatic test methods used (43) have been studied from the standpoint of dose-response curves and an "activity coefficient" has been determined (44). Dosage response curves are obtained by a modification of the agar cup plate technique. As a standard for comparison, a 5 mm. zone at 1% concentration of the compound tested was arbitrarily chosen as the activity 1. Using this procedure, fungistatic agents can be compared on a numerical basis and be given numerical values. This constitutes a significant and valuable development in the field of fungistatic testing methods.

Relative fungicidal activity has also been determined by a modification of the Burlingame and Reddish method referred to above. The modifications include use of fifteen-day-old fungus cultures, culture-fungicide contact of one minute, use of serum in the culture medium, and use of an acetone-water mixture for the washing phase of the test procedure (45). By the use of this modification it is possible to accurately classify fungicides by their minimal fungicidal concentration so that the relative merits of fungicidal compounds can readily be determined.

The accuracy of these methods in evaluating fungicidal and fungistatic activity has recently been demonstrated in a study of the antifungal properties of quinones and quinolines (46). These studies demonstrate again the value of these methods for the purpose. They are highly recommended for use as standard methods for the evaluation and control of fungicidal and fungistatic products.

Antiseptic Ointments

The F. D. A. method for testing liquid antiseptics described earlier is employed for testing products which are applied clinically for short time periods—such as in first aid, preoperative skin preparation, oral use, etc. Antiseptic ointments, on the other hand, are tested by an entirely different method since they customarily are applied for long periods of time. Because such antiseptics are in continuous contact with the bacteria present, they need only be bacteriostatic in order to prove effective in rendering the organisms innocuous.

The laboratory test for antiseptic ointments is therefore quite different from the test for liquid antiseptics. The standard method employed is known as the F. D. A. Serum Agar Plate Method (3) (6). This method, briefly, is as follows:

Nutrient agar containing 10% normal horse serum is inoculated with 0.1 cc of a 24-hour culture of *Staphylococcus aureus* (of standard resistance), mixed thoroughly, poured into a sterile 9 cm Petri dish and allowed to harden. The ointment being tested is softened and applied to the surface of the agar in a narrow streak, by means of a glass rod. Melted vaseline is streaked on another portion of the plate to serve as a control. The plates are incubated at 37°C. for 48 hours and observed for the presence of a clear zone around the ointment.

If the preparation tested is antiseptic, a clear zone will completely surround the streaked ointment. The width of the clear zone will indicate the antiseptic activity of the product being tested and show whether or not the product is counteracted by blood serum. There should, of course, be no clear zone around the vaseline control.

"The size of the zone of inhibition given by this method is important and has a direct bearing on the clinical effectiveness of the ointment being tested. Since Ammoniated Mercury Ointment, U. S. P., has been widely and successfully used as an antiseptic ointment by the medical profession and others for a great many years, it is used as the standard of comparison in the test for antiseptic ointments described above. A clear zone of inhibition of at least 0.2 cm. in width and 0.2 cm. in depth is given by this standard antiseptic ointment (47). Antiseptic ointments tested by this method must therefore give a minimum zone of 0.2 cm. if they

are to be considered effective antiseptic ointments, although many ointments give a clear zone of inhibition greater than 0.2 cm. This test simulates actual conditions of use to some degree, since the semisolid serum-agar is somewhat comparable to wound exudate and tissue" (48).

Antiseptic Wet Dressings, Dyes, Powders, etc.

When antiseptics used as wet dressings, as well as antiseptic dyes, powders, etc., are employed clinically, they remain in contact with the bacteria present for long periods of time. The standard method of test is therefore designed to determine bacteriostatic activity, not germicidal effect as is done with liquid antiseptics for general use. The standard testing procedure is similar to that just described for antiseptic ointments, except that the preparation being tested is applied in a 1.5 cm. cup made in the agar. This method is known as the "F. D. A. Serum-Agar Cup Plate Method" (3) (6).

The interpretation of the results obtained with these preparations is comparable to that given above for antiseptic ointments except that the acceptable zone of inhibition is somewhat larger. An antiseptic dye solution of known merit, merbromin (Mercurochrome), which has been widely and successfully used in surgery and first aid, gives a zone of 0.7 to 0.9 cm. by the method described above (49). Hence in order to pass this test, these products must give a zone of at least 0.7 cm.

While the serum-agar cup plate method does not duplicate practical conditions of use, it may be employed properly to compare antiseptic preparations of known clinical value. Furthermore, the test does provide a means of measuring the penetration and inhibitory action of such antiseptics in the presence of blood serum. This test like other laboratory tests should be followed by practical and clinical studies (48).

Summary

The purpose of this review has been to bring present information up to date on standard methods of testing liquid antiseptics intended for general use, antiseptic ointments, antiseptics used for wet dressings, and fungicides. In addition, a number of the more recently proposed methods of test have been discussed.*

* A more general review of the whole field of disinfection and antisepsis has recently been made by Klarmann. (50)

Although many of these proposed tests were devised for the purpose of simulating actual conditions of use, none have been able to meet this commendable objective. The so-called "toxicity index" tests are excellent examples, for not one of these tests can be interpreted upon a practical basis. They (1) fail to test against a representative tissue, (2) use improper dilutions of antiseptic, and (3) false evaluations are derived from results of these tests.

Despite the fact that many of the proposed newer tests are of academic interest, manufacturers and users of antiseptics are more interested in such practical considerations as positive action against organisms encountered in body areas on or in which antiseptics are used, and in relative non-toxicity of antiseptics in the dilutions employed.

A standard test method for antiseptics should be based upon known values of proved merit. It should accurately determine ability to kill or inactivate the most resistant organism commonly found in areas treated by means of antiseptics. The test *itself* should contain a safety factor so that users may rely upon the efficacy of the antiseptic. It should classify antiseptics upon a sound clinical basis. And finally, test conditions should be such that they may be duplicated exactly, wherever and whenever the test is conducted.

The official F. D. A. method of testing liquid antiseptics is the *only* test proposed to date that satisfactorily meets every one of these conditions. In use since 1926 and established as an official test since 1931, the F. D. A. method has made it possible to standardize antiseptics upon a sound basis. As a result, the field of antiseptics is so effectively controlled that all users may now be assured of efficacious products.

New methods of test for antiseptics are constantly being proposed. All of us sincerely hope that some day we will be provided with the ideal test. But in the meantime, manufacturers of antiseptics may take comfort from the knowledge that the official F. D. A. test now in use is a *good* test—one that is based upon sound laboratory and clinical evidence.

REFERENCES

1. Reddish, G. F. Recent Developments in Methods of Testing Germicides. *Ind. and Eng. Chem., 10,* 425-427 (1938).

2. Reddish, G. F. Editorial. *J. Am. Pharm. Assoc., 16,* 501-2 (1927).

3. Reddish, G. F. Methods of Testing Antiseptics. *J. Lab. Clin. Med., 14,* 649-658 (1929).

4. Federal Food, Drug, and Cosmetic Act of June 25, 1938, Chapter II, Sec. 201.

5. Patterson, A. M. Meaning of "Antiseptic," "Disinfectant," and Related Words. *Amer. Jour. Public Health, 22,* 465-472 (1932).

6. U. S. Food and Drug Administration Methods of Testing Antiseptics and Disinfectants. Circular 198, U. S. Dept. of Agriculture, 1931.

7. Reddish, G. F. The Resistance to Phenol of Staphylococcus Aureus. *Amer. Jour. Public Health, 15,* 534-538 (1925).

8. Reddish, G. F. The Effect of Peptone on the Resistance of Staphylococcus aureus. *Jour. Amer. Pharm. Assoc., 27,* 331-334 (1938).

9. Rideal, S., and Walker, J. T. A. Examination of Disinfectants. *J. San. Inst., 24,* 424-441 (1903); Approved Technique of the Rideal-Walker Test, London, 1921.

10. Hygienic Laboratory Bulletin No. 82, 1912; *Public Health Reports,* Reprint No. 675, 1921.

11. Reddish, G. F. Determining the Germicidal Efficiency of Disinfectants. *Am. J. Pub. Health, 16,* 283-286 (1926).

12. Reddish, G. F. Examination of Disinfectants. *Am. J. Public Health, 17,* 320-329 (1927).

13. Reddish, G. F. The Significance of the Phenol Coefficient. *Soap and Sanitary Chemicals, 11,* 95-113 (1935).

14. Reddish, G. F. Limitations of the Phenol Coefficient. *Ind. and Eng. Chem., 29,* 1044-1047 (1937).

15. Reddish, G. F. Methods of Testing Antiseptics. *Drug Markets,* May 3, 1927.

16. Varley, J. C., and Reddish, G. F. The Phenol Coefficient as a Measure of the Practical Value of Disinfectants. *J. Bact., 32,* 215-225 (1936).

17. Reddish, G. F. The Standardization of Disinfectants and Antiseptics. *The Newer Knowledge of Bacteriology and Immunology,* Chapter 22. Jordan & Falk. University of Chicago Press, 1928.

18. Reddish, G. F. Antiseptics: A Comparative Study of Laboratory and Practical Tests. *J. Amer. Pharm. Assoc., 25,* 1117-1121 (1936).

19. Simmons, J. S. Bactericidal Action of Mercurochrome-220 Soluble and Iodine Solutions in Skin Disinfection. *J. A. M. A., 91,* 704-708 (1928).

20. Reddish, G. F., and Drake, W. E. Mercurochrome-220 Soluble and Tincture of Iodine: Comparison of Germicidal Efficiency in Skin Disinfection. *J. A. M. A., 91,* 712-716 (1928).

21. Hunt, G. A. The Use of Cutaneous Staphylococcus Lesions in Mice for the Evalution of the Germicidal Activity of Disinfectants. *J. Inf. Dis., 60,* 232-237 (1937).

22. Nungester, W. J., and Kempf, A. H. An "Infection-Prevention" Test for the Evaluation of Skin Disinfectants. *J. Inf. Dis., 71,* 174-178 (1942).

23. Price, P. B. The Bacteriology of Normal Skin; A New Quantitative Test Applied to a Study of the Bacterial Flora and the Disinfectant Action of Mechanical Cleansing. *J. Inf. Dis., 63,* 301-318 (1938).

24. Price, P. B. Ethyl Alcohol as a Germicide. *Arch. Surg., 38,* 528-542 (1939).

25. Salle, A. J., McAnnie, W. A., and Schechmeister, J. L. A New Method for the Evaluation of Germicidal Substances. *J. Bact., 34,* 267-273 (1937).

26. Nye, R. N. The Relative in Vitro Activity of Certain Antiseptics in Aqueous Solution. *J. A. M. A., 108,* 280-287 (1937).

27. Welch, H., and Hunter, A. C. Method of Determining the Effect of Chemical Antisepsis on Phagocytosis. *Amer. J. Pub. Health, 30,* 129-137 (1940).

28. Welch, H. Mechanism of the Toxic Action of Germicides on Whole Blood Measured by the Loss of Phagocytic Activity of Leucocytes. *J. Immunol., 37,* 525 (1939).

29. Welch, H., and Brewer, C. M. Relative Toxicity of Certain Antiseptics Containing Soap and Alcohol: With Special Reference to Mouth Washes. *Am. J. Pub. Health, 32,* 261-267 (1942).

30. Welch H., Slocum, G. G., and Hunter, A. C. Method of Determining the Toxicity of Antiseptics as Measured by the Destruction of Human Leucocytes. *J. Lab. and Clin. Med., 27,* 1432-1438 (1942).

31. Shippen, L. P. A Fallacy in the Standard Methods of Examining Disinfectants. *Am. J. Pub. Health, 18,* 1231-1234 (1928).

32. Brewer, J. H. The Antibacterial Effects of the Organic Mercurials. *J. A. M. A., 112,* 2009-2018 (1939).

33. Brewer, J. H. Clear Liquid Medium for the "Aerobic" Cultivation of Anaerobes. *J. A. M. A., 115,* 598-600 (1940).

34. Marshall, M. S., Gunnison, J. B., and Luxen, M. P. Test for the Sterility of Biologic Products. *Proc. Soc. Exp. Biol. Med., 43,* 672-673 (1940).

35. Natl. Inst. of Health Circ. Culture Media for Sterility Tests, Jan. 15, 1945.

36. Sarber, R. W. An in Vivo Method for the Evaluation of Germicidal Substances Used for Skin Disinfection. *J. Pharmacol. Exp. Ther., 75,* 277-281 (1942).

37. Green, T. W., and Birkeland, J. M. The Use of the Developing Chick Embryo as a Method of Testing the Antibacterial Effectiveness of Wound Disinfectants. *J. Inf. Dis., 74,* 32-36 (1944).

38. Salle, A. J., and Catlin, B. W. Profile Evaluation of Germicides. *J. Amer. Pharm. Assoc., 36,* 129-133 (1947).

39. Ostrolenk, M., and Brewer, C. M. A Bactericidal Spectrum of Some Common Germicides. Proc. of Meetings, *Soc. Amer. Bact., 1,* 28 (1948).

40. Morton, H. E., North, Jr., L. L., and Engley, Jr., F. B. The Bacteriostatic and Bactericidal Actions of Some Mercurial Compounds on Hemolytic Streptococci. *J. A. M. A., 136,* 37-41 (1948).

41. Brewer, J. H. Reduction of Infectivity of Certain Pathogenic Bacteria by "Mercurochrome." *J. A. M. A., 137,* 858-861 (1948).

42. Powell, H. M. Antibacterial Action of "Merthiolate" Against Hemolytic Streptococci. *J. A. M. A., 137,* 862-864 (1948).

43. Burlingame, E. M., and Reddish, G. F. Laboratory Methods for Testing Fungicides Used in the Treatment of Epidermophytosis. *J. Lab. Clin. Med., 24,* 765-772 (1939).

44. Oster, K. A., and Golden, M. J. Studies on Alcohol-Soluble Fungistatic and Fungicidal Compounds. I. Evaluation of Fungistatic Laboratory Test Methods. *J. Amer. Pharm. Assoc., 36,* 283-288 (1947).

45. Golden, M. J., and Oster, K. A. Studies on Alcohol-Soluble Fungistatic and Fungicidal Compounds. II. Evaluation of a Fungicidal Laboratory Test Method. *J. Amer. Pharm. Assoc., 36,* 359-362 (1947).

46. Oster, K. A., and Golden, M. J. Studies on Alcohol-Soluble Fungistatic and Fungicidal Compounds. III. Evaluation of the Antifungal Properties of Quinones and Quinolines. *J. Amer. Pharm. Assoc., 37,* 429-434 (1948).

47. Reddish, G. F. Antiseptic Action of U. S. P. and N. F. Ointments. *J. Amer. Pharm. Assoc., 18,* 576-578 (1929).

48. Reddish, G. F. Interpretation of Results Obtained in Testing Disinfectants, Antiseptics, and Fungicides. *Bull. Natl. Formulary Com., 15,* 81-90 (1947).

49. Reddish, G. F. The Stability of Mercurochrome Solutions. *J. Lab. Clin. Med., 13,* 859-861 (1928).

50. Klarmann, E. G. Disinfection and Antisepsis: Trends and Ideas. *Amer. Jour. Pharm., 120,* 321-355, 367-385 (1948).

SELECTED ABSTRACTS

Pethidine and Scopolamine in Labor. H. Roberts. *Brit. Med. J. No. 4577:*590 (1948). Pethidine was first introduced as a synthetic substitute for atropine but it was soon found to have analgesic as well as spasmolytic properties. Thus it was introduced to labor analgesia but it did not give consistent results. The concurrent administration of scopolamine has increased the effectiveness of pethidine. Scopolamine occasionally causes excitement in the mothers and respiratory depression in the infant but its usefulness outweighs these disadvantages.

In a series of 500 primipara and multipara patients the first dose of medication was given intramuscularly after labor was established. The dose consisted of 100 mg. of pethidine and 1/150 gr. of scopolamine. The dose was repeated one hour later if necessary and then at intervals of 4 to 6 hours. Scopolamine should not be given within 2 hours of delivery, if possible to prejudge. The maximum dosage given in this series was in a labor lasting 33 hours. A total of 500 mg. of pethidine and 1/50 gr. of scopolamine were given. The medication was given intravenously, well diluted, over a period of at least 3 minutes if the patient was well advanced in labor upon admission. If necessary gas was given during delivery.

Complete or almost complete relief was obtained in 82.2 per cent, relief to the point of an easy labor in 13.8 per cent, and slight relief in 3.6 per cent of the patients. The patients were able to cooperate in the majority of cases, there was no increase in the length of labor, and the fetal mortality rate was not increased. The author states that the patients as well as the nursing staff were enthusiastic about this method for the relief of pain of childbirth.

Bacteriological Properties of Aureomycin. T. F. Paine, Jr., H. S. Collins, and M. Finland. *J. Bacteriol. 56:*489 (1948). A study of the bacteriological action and properties of aureomycin hydrochloride *in vitro* has shown that the antibiotic is effective against

a wide range of gram-positive and gram-negative bacteria. Among the 186 strains of bacteria isolated from various patients only *Proteus vulgaris* and *Pseudomonas aeruginosa* exhibited relatively high and consistent resistance to the antibiotic. Against various cocci aureomycin was found to be less effective than penicillin but more effective than streptomycin, weight for weight. Against gram-negative bacilli it was about as effective as streptomycin.

Various factors which influence the concentration of the antibiotic required for complete inhibition of bacteria were found to be the number of organisms present, the phase of their growth, and the pH of the test media. Resistance to aureomycin was not encountered to any marked degree in most of the organisms studied. Clinical trial to date had also indicated that the development of resistance to aureomycin was not a great problem with which to contend. No evidence was found to indicate that any aureomycin inhibiting substance similar to penicillinase was developed.

In sealed ampuls as a dry powder the antibiotic was stable for at least 6 months. In high concentrations in distilled water it was also stable for periods up to 2 or 3 weeks at both 4°C. and 37°C. However, in low concentrations in the presence of broth, plasma, or in blood agar it loses potency rapidly. pH had a marked effect on the antibacterial activity in that alteration from the acid to alkaline range decreased the activity. In contrast to this streptomycin is more active in the alkaline range.

Sucessful Treatment of Guinea Pigs With Tuberculosis With Potassium Iodide and Streptomycin. E. Woody, Jr., and R. C. Avery. *Science 108:*501 (1948). Clinical observations in the past have shown that tubercle bacilli will appear in the sputum of tubercular patients following the administration of potassium iodide. This suggested the possible value of potassium iodide in the treatment of tuberculosis.

The authors inoculated four groups of guinea pigs with tubercle bacilli. One group acted as controls and the other 3 groups were treated, respectively, with potassium iodide, streptomycin, and potassium iodide-streptomycin. Treatment was begun 21 days after inoculation. The dosage of potassium iodide was 80 mg. per Kg.

of body weight per day, given as a weak aqueous solution by stomach tube. The streptomycin was given intramuscularly on the basis of 12,500 micrograms per Kg. of body weight per day. Following 4 weeks of treatment all animals were killed and autopsied. Gross findings showed tuberculous infections in all the viscera of the controls and KI-treated animals. In the streptomycin-treated group half the animals showed spread of the infection to the organs, and in the streptomycin-KI-treated group there was no infection in the organs.

A second series of tests were performed to determine survival. Three groups of guinea pigs were used, one acted as controls, one received three times the previous dosage of streptomycin, and the third group received three times the streptomycin combined with KI as before. Treatment was begun four weeks after inoculation and continued for five weeks. At the end of twelve weeks 13 of 13 controls were dead, 6 of 13 in the streptomycin group, and only 2 of 14 in the streptomycin-KI group.

The authors stated that clinical trials were in progress at the time of writing to further investigate the value of this therapy.

The Value of Paludrine as an Antimalarial. (Editorial.) *Brit. Med. J. No. 4593:*106 (1949). When paludrine first appeared it was hoped that the solution to all the problems of the treatment of malaria had been found. However, it was soon apparent that although acute attacks due to *Plasmodium vivax* were controlled by the drug it did not eradicate the infection. It also cannot be relied upon as a true causal prophylactic. In cases of infection due to *Plasmodium falciparum* the results have been varied and confusing. Reports published concerning studies made on various strains from various parts of Africa, Asia, Europe, and New Guinea indicate that the results obtained vary with the locale in many instances. Other evidence also indicates that there are probably different strains of *P. falciparum*, which, in part, at least, accounts for the varying results from the use of paludrine. Evidence is also growing that paludrine produces resistant strains of various plasmodia comparatively easily. Resistance against quinine and quinacrine seems to be very difficult to produce.

Paludrine has a low toxicity but there is evidence that prolonged use of the drug may cause loss of appetite, reduction in weight, and lack of energy. It has been shown that doses of 900 mg. of paludrine reduces the acidity and volume of gastric juice.

A comparison of the effects of paludrine and quinacrine on the prophylaxis and treatment of falciparum malaria in West Africa revealed that as a prophylactic in doses of 100 mg. a day quinacrine has practically no failures but the same dose of paludrine has caused a number of failures. Quinacrine is not a true causal prophylactic but if continued as a suppressive will bring about a radical cure. Paludrine is a true causal prophylactic for some strains but does not produce a radical cure. Quinacrine has no effect on gametocytes while paludrine inhibits their development in the mosquito. Thus it is felt that the ideal drug for the treatment of prophylaxis of malignant tertian malaria has not yet been found.

Intravenous administration of antimalarials is fortunately rarely necessary. However, in cerebral emergencies and in patients who are unable to swallow or who have gastro-intestinal complications it may be necessary. Paludrine acetate has been administered intravenously in doses ranging from 25 to 400 mg. with only a mild phlebitis of the injected vein as an untoward reaction. More recently the acetate has been replaced by the more soluble lactate for intravenous use.

BOOK REVIEWS

The Pharmaceutical Pocket Book, Fifteenth Edition. Published by direction of the Council of the Pharmaceutical Society of Great Britain. 427 pages incl. index. The Pharmaceutical Press, 17 Bloomsbury Square, London W. C., 1948. Price 12s 6d postage 4d.

This pocket size volume is a worthy member of that group of books published by the Pharmaceutical Press. It is intended to serve students of pharmacy in outlining some of the basic principles upon which the profession is based and also to serve as a useful reference for those in practice.

The scope of the book is as follows:

(a) Activities of the Pharmaceutical Society of Great Britain.

(b) The Science and Art of Pharmacy (a section dealing with the scope of pharmacy and the fields of science covered by it).

(c) Dispensing—ointment bases, sterilization and preparation of injections.

(d) Equivalent Formulae and Doses of Official (B. P.) Medicaments.

(e) Forensic Pharmacy.

(f) Poisoning (including antidotes).

(g) Biochemical Analyses.

(h) Notes on Bacteriology.

(i) Food and Diet.

(j) The Endocrines.

(k) Veterinary Dose Table.

(l) Synonyms and Trade Names.

From a review of the various sections of this book it would appear to be a very useful reference even for pharmacists in the United States. It is particularly valuable for those who wish to learn something of the organization and training of pharmacists in Great Britain. Many practical points are given the reader and this little volume is a worthy companion of that highly regarded two volume work, "The Extra Pharmacopoeia." No pharmacist can have too many sources of information and this "Pocket Book" is quite worthwhile.

Biochemical Preparations. Vol. I. By Herbert E. Carter. 76 pages incl. index. John Wiley & Sons, Inc., New York. 1949. Price $2.50.

This is the first volume of a planned series of similar volumes dealing with the preparation of chemicals of biochemical interest. The style and manner of presentation is similar to that used in the well known work "Organic Syntheses" now in its twenty-seventh volume.

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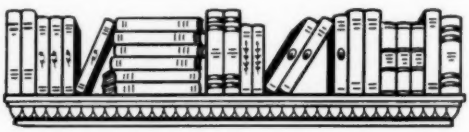
It is believed that this series should prove a most useful one to both biochemists and teachers, saving considerable time which might otherwise be spent in searching for a suitable method by reference to the very voluminous literature.



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250 "	5.25	9.00	18.75	20.00	250 " 10.25

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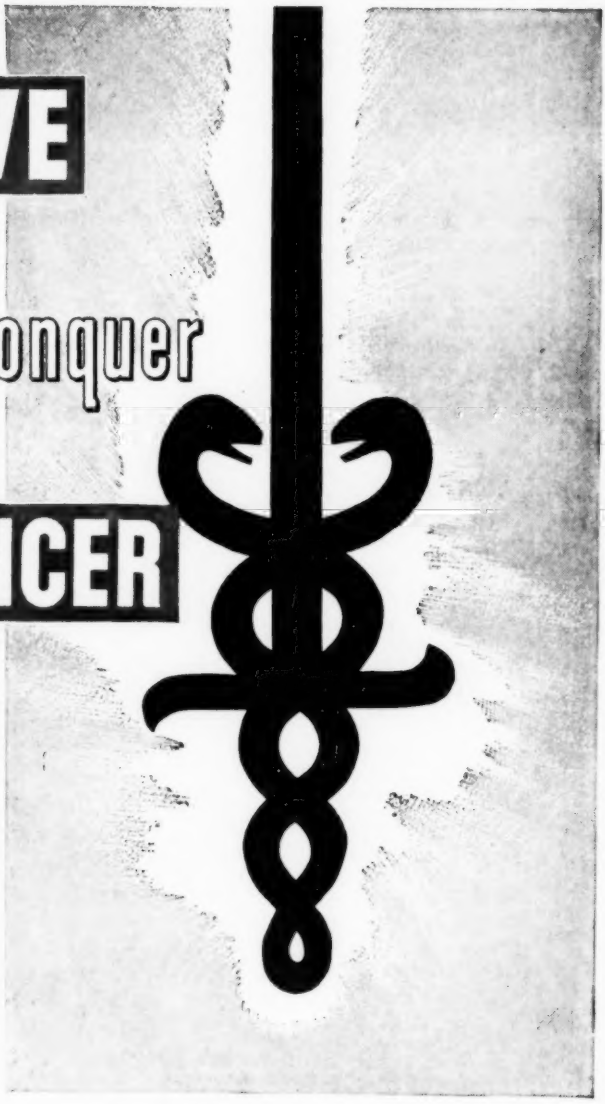
to conquer

CANCER



JUST MAIL IT TO "CANCER"

Just write "CANCER" on the envelope containing your check or money order. It will be delivered to the American Cancer Society office in your state.

GIVE - *as generously as you can*

AMERICAN CANCER SOCIETY